PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 12364890	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/AU2003/001544**	International Filing Date *(day/month/year)* 18 November 2003	Priority Date *(day/month/year)* 18 November 2002
International Patent Classification (IPC) or national classification and IPC **Int. Cl.**[7] C12Q 1/68		
Applicant MURDOCH CHILDRENS RESEARCH INSTITUTE et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of **6** sheet(s).

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [X] Certain observations on the international application

Date of submission of the demand 1 June 2004	Date of completion of the report 7 March 2005
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **ALISTAIR BESTOW** Telephone No. (02) 6283 2450

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/001544

I. Basis of the report

1. With regard to the elements of the international application:*

☐ the international application as originally filed.

☒ the description, pages **1 - 95** as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☒ the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages **96 - 101** received on **17 December 2004** with the letter of **17 December 2004.**

☒ the drawings, pages **1/11 – 11/11** as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☒ the sequence listing part of the description:
pages **1 - 17** as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1 – 20	YES
	Claims 21 – 25	NO
Inventive step (IS)	Claims	YES
	Claims 1 – 25	NO
Industrial applicability (IA)	Claims 1 - 25	YES
	Claims	NO

2. Citations and explanations (Rule 70.7)

The following citations, which were cited in the International Search Report, are relevant for the purposes of novelty and/or inventive step.

D1. DeRisi J (2000). Unit 22.1: Overview of nucleic acid arrays. In: Current Protocols in Molecular Biology. Supplement 49, pp 22.1.1–22.1.3. John Wiley & Sons, Inc.
D2. Hone S and Smith R (2002). Otolaryngologic Clinics of North America 35(4): 751–764.
D3. WO200250305 A1 20 December 2000.
D4. Dong J et al (2001). Molecular Genetics and Metabolism 73(2): 160–163 (abstract).
D5. Bacino C et al (1995). Pharmacogenetics 5(3): 165–172 (abstract).
D6. Kenna M et al (2001). Archives of Otolaryngology — Head & Neck Surgery 127(9): 1037–1042 (abstract).
D7. Wiszniewski W et al (2001). Genetic Testing 5(2): 147–148 (abstract).
D8. Pampanos A et al (2002). International Journal of Pediatric Otorhinolaryngology 65(2): 101–108 (abstract).
D9. Scott D et al (2000). Human Molecular Genetics 9(11): 1709–1715 (abstract).
D10. Dreyer B et al (2001). American Journal of Human Genetics 69(1): 228–234 (abstract).
D11. Chen Z-Y and Corey D (2002). Journal of Neurobiology 53: 276–285.
D12. Database Accession # AC026202. Chen C et al (18 October 2002). Homo sapiens chromosome 3 clone RP11-572B2 map 3p, complete sequence.

Novelty (N) and Inventive Step (IS)

D1 presents an overview of the various uses of nucleic acid arrays, including the use of oligonucleotide arrays for genotyping. D1 does not discuss the genotyping of a subject with respect to connexion 26, pendrin, mitochondrial 125 rRNA or usherin, as specified in the claims. Therefore all of the claims are novel in view of D1. It would not be obvious from D1 to perform the genotyping specified in the claims, and therefore the claims are inventive in view of D1.

D2 presents a review of genetic testing for the evaluation of pediatric hearing loss. This review states that *'screening for mutations in connexin 26 has become increasingly available in many centers and should be performed in all cases of nonsyndromic hearing loss'*. In particular, the review cites the role of the connexin 26 mutations 35delG,167delT and 235delC in genetic screening for deafness. The review also discloses the need for further mutational screening, including in pendrin, for deafness. Such genotyping is so commonly undertaken using oligonucleotide microarray technology that such a method is considered inherent to the disclosure of the citation. D2 does not specify the particular hybridisation conditions referred to in the claims, or the particular oligonucleotides of claims 21 - 25 and therefore the claims are novel. However, there is nothing inventive in the hybridisation conditions specified in the claims, these being very standard in the art.

(continued in Supplemental Box)

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Descriptive support

Claims 21–25 are not fully supported by the description. The applicant's invention resides in the provision of methods of genotyping for deafness by immobilized allele-specific oligonucleotide hybridization. In contrast, however, these claims merely recite oligonucleotides *per se*, and do not recite methods for their use in genotyping for deafness. Therefore, in the absence of an inclusion in the claims of methods for the use of these oligonucleotides, the claims are not fully supported by the description.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of V

Neither has there been shown any inventive merit in using immobilised oligonucleotides SEQ ID 1 – 64 to perform the genotyping, or the sequences themselves, so whilst these may not have been specifically disclosed in D2, their use is not inventive in view of D2. Therefore none of the claims are inventive in view of D2.

D3 discloses the use of immobilized oligonucleotide microarrays for genotyping the connexin 26 35delG mutation associated with deafness. For similar reasons as provided for D2 above, none of the claims show any inventive merit in view of D3

D4 and D5 (amongst other citations in this field of work) each disclose the use of allele-specific oligonucleotide hybridization for the genotyping of deafness. In particular, these citations disclose the identification of the connexin 26 167delT and 35delG mutations (D2) and the mitochondrial 125 rRNA A1555G mutation. These mutations are specifically detected by the oligonucleotides of the claims. For similar reasons as provided for D2 above, none of the claims show any inventive merit in view of D3.

D6 – D10 each disclose allelic mutations associated with deafness. In particular, these citations disclose the connexin 26 mutations 35delG (D5–D7), M34T (D5, D6), L90P (D5, D7), 167delT (D5), V37I (D5), R143W (D5), 313del14 (D6) ad W24X (D7), the pendrin mutations L236P, T416P and E384S (all D8) and the usherin mutation 2299delG (D9). These citations do not disclose methods for genotyping based upon analysis of these mutations, or the specific conditions of hybridisation noted in the claims, and therefore claims 1 – 20 are considered to be novel in the light of these citations. However, these citations disclose the oligonucleotides of claims 21 – 25, and therefore these claims lack novelty. For similar reasons as provided for D2 above, none of the claims show any inventive merit in view of D3.

Furthermore, the consideration of either D1, D2, D3 or D11 in conjunction with any one of D4 – D10 would lead the skilled addressee to the subject matter of the claims, namely the use of specific mutations for genotyping for deafness using immobilized oligonucleotide arrays, and therefore the claims lack inventive step in the light of these combinations of documents.

D12 provides no suggestion as to use or selection of subsequences suitable for genotyping use, and therefore the claims are considered inventive in the light of this citation.

Industrial Applicability (IA)

Claims 1 - 25 meet the requirements of the PCT with regard to Industrial Applicability.

CLAIMS:

1. A method for genotyping a subject with respect to a gene or target nucleic acid sequence selected from connexion 26, pendrin, mitochrondrial 125 rRNA or ussherin associated with a pathological condition, said method comprising contacting an allele specific oligonucleotide immobilized to a solid support with a single-stranded form of RNA or DNA from a subject to be tested labeled directly or indirectly with a reporter molecule capable of giving an identifiable signal under conditions which comprise hybridization in the presence of 1-4 X SSC at 30-50°C for 15-90 min followed by washing at 30-50°C in the following sequence:

1-4 X SSC/0.05% - 0.4% SDS (1-5 min);
0.1-1 X SSC/0.05% - 0.4% SDS (2-10 min);
0.5 X -5 X SSC (0.5-3 min);
2-8 X SSC/0.05% (0.5-3 min); and
2-8 X SSC/0.05%-2% Tween (0.5-3 min).

which permit hybridization of single stranded RNA or DNA which is exactly complementary to the immobilized allele specific oligonucleotide but substantially less or no hybridization of non-complementary single-stranded RNA or DNA molecules and then screening for the presence or absence or level of reporter molecule which provides an indicator of the genetic identity of the single-stranded RNA or DNA molecule which in turn provides the genotype of the subject.

2. The method of Claim 1 wherein the RNA or DNA from the test subject is directly labeled with labeled nucleotides incorporated *via* polymer chain reaction (PCR).

3. The method of Claim 1 wherein the RNA or DNA from the test subject is indirectly labeled with labeled nucleotides *via* hybridization of a labeled oligonucleotide to the test RNA or DNA.

4. The method of Claim 1 wherein the subject is selected for a human, a non-human primate, a livestock animal, a laboratory test animal, a companion animal and a captured

wild animal.

5. The method of Claim 1 wherein the subject is a human.

6. The method of Claim 5 wherein the pathological condition is selected from an autoimmune disease, inflammatory condition, cancer, neurological disorder and a neurodegenerative disorder.

7. The method of claim 1 wherein the pathological condition is genetic deafness or a propensity for development of genetic deafness.

8. The method of Claim 1 wherein the pathological condition is associated with genetic deafness.

9. The method of Claim 1 wherein the hybridization step is under differential hybridization conditions which permits differential hybridization between identical nucleotide sequences and sequences having at least one mismatch and the identity of the genotype of the subject is determined by the presence, absence or level of signal from the reporter molecule.

10. The method of Claim 1 wherein the immobilized oligonucletides are from about 5 to about 100 nucleotides in length.

11. The method of Claim 12 wherein the immobilized oligonucleotides are from about 10 to about 30 nucleotides in length.

12. The method of Claim 12 wherein the immobilized oligonucleotides are from about 15 to about 30 nucleotides in length.

13. The method of Claim 12 wherein the immobilized oligonucleotides are selected from SEQ ID NO:1 to 64.

14. The method of claim 1 wherein a sequence of nucleotides is interrupted up- or down-stream of the immobilized oligonucleotide to improve hybridization sensitivity.

15. The method of Claim 16 wherein the interruption is in a sequence of G residues.

16. A method for genotyping a human subject from a gene or nucleic acid target selected from *connexion 26, pendrin*, mitochondrial 12S rRNA and *usherin* wherein a mutation in one or more of these genes or targets is indicative of genetic deafness or a propensity to develop genetic deafness, said method incorporating a label directly or indirectly into genomic DNA amplified from the human subject to be tested using primers which flank a DNA sequence corresponding to a potential mutation in a gene or nucleic acid target listed above and contacting single-stranded labeled forms of the amplified DNA with an immobilized oligonucleotide selected from SEQ ID NO:1 to SEQ ID NO:64 under stringency conditions such that substantially only identically complementary DNA from the subject is capable of hybridizing to the corresponding immobilized oligonucleotide and screening for hybridization by measuring a signal or level of signal from the label.

17. A method for genotyping a human subject from a gene or nucleic acid target selected from *connexion 26, pendrin*, mitochondrial 12S rRNA and *usherin* wherein a mutation in one or more of these genes or targets is indicative of genetic deafness or a propensity to develop genetic deafness, said method incorporating a label into genomic DNA amplified from the human subject to be tested using primers which flank a DNA sequence corresponding to a potential mutation in a gene or nucleic acid target listed above and contacting single-stranded labeled forms of the amplified DNA with an immobilized oligonucleotide selected from SEQ ID NO:1 to SEQ ID NO:32 under stringency conditions of 1-4 X SSC at 30-50°C for 15 min to 90 min followed by washing at 30-50°C in the following sequence:-

1-4 X SSC/0.05% - 0.4% SDS (1-5 min);
0.1-5 X SSC/0.05% - 0.4% SDS (2-10 min);

0.5 X -5 X SSC (0.5-3 min);
2-8 X SSC/0.05% (0.5-3 min); and
2-8 X SSC/0.05%-2% Tween (0.5-3 min);

such that substantially only identically complementary DNA from the subject is capable of hybridizing to the corresponding immobilized oligonucleotide and screening for hybridization by measuring a signal or level of signal from the label.

20. A method for genotyping a human subject from a gene or nucleic acid target selected from *connexion 26, pendrin*, mitochondrial 12S rRNA and *usherin* wherein a mutation in one or more of these genes or targets is indicative of genetic deafness or a propensity to develop genetic deafness, said method incorporating a label into genomic DNA amplified from the human subject to be tested using primers which flank a DNA sequence corresponding to a potential mutation in a gene or nucleic acid target listed above and contacting single-stranded labeled forms of the amplified DNA with an immobilized oligonucleotide selected from SEQ ID NO:1 to SEQ ID NO:32 under stringency conditions of 1-4 X SSC at 30-50°C for 15 min to 90 min followed by washing at 30-50°C in the following sequence:-

1-4 X SSC/0.05% - 0.4% SDS (1-5 min);
0.1-6 X SSC/0.05% - 0.4% SDS (2-10 min);
0.5 X -5 X SSC (0.5-3 min);
2-8 X SSC/0.05% (0.5-3 min); and
2-8 X SSC/0.05%-2% Tween (0.5-3 min);

such that substantially only identically complementary DNA from the subject is capable of hybridizing to the corresponding immobilized oligonucleotide and screening for hybridization by measuring a signal or level of signal from the label, wherein a GI value is determined by the algorithm:-

$$GI = \frac{SV_N}{SV_N + SV_M}$$

wherein:

SV_N is the normal spot value; and
SV_M is the mutant spot value;

such that:

if $0.8 < GI < 1.0$, then the genotype is N/N;
if $0.65 < GI < 0.5$, then the genotype is N/M; and
if $0.0 < GI < 0.2$, then the genotype is M/M;

wherein:

N is a normal allele; and
M is a mutant allele.

21. A set of one or more oligonucleotides having the sequence:-

$$[n]_x - A$$

wherein:

n is one or a range of different nucleotides;
x is the length of the nucleotide sequence [n]; and
A is a nucleotide sequence selected from SEQ ID NOs:33 to 64.

22. The set of one or more oligonucleotides of claim 21 wherein n is T.

23. The set of one or more oligonucleotides of claim 21 or 22 wherein x is from about 5 to about 30.

24. The set of one or more nucleotides of claim 21 wherein $[n]_x - A$ is selected from SEQ ID Nos: 1 to 32.

25. A kit comprising one or more oligonucleotides of any one of claims 21 to 24.